REMARKS/ARGUMENTS

Claims 9-16 and 26 are pending in the present application. Withdrawn claims 17-23 are cancelled with this amendment. Claims 9-14 stand rejected for allegedly being obvious over McElroy *et al.* in view of a number of references that allegedly teach the stable transformation of barley plants. Claims 15 and 16 stand rejected over McElroy *et al.* in view of the same references plus Perera *et al.* which discloses the use of *cod A* as a negative selectable marker. As explained below, this rejection is respectfully traversed.

New claim 26 is added with this amendment. Support for this claim is found, for example, in the last full paragraph of page 26 of the specification. In addition, claim 9 has been amended to clarify that the excision of the *Ds* element is mediated by a ***transposase***, rather than a transposon. Support is replete throughout the specification. Finally, claim 10 has been cancelled without prejudice. Applicants specifically reserve the right to pursue all the subject matter of the unamended claims in one or more subsequent applications.

As explained in previous responses, the Examiner has not established a proper case of *prima facie* obviousness. A *prima facie* case of obviousness requires that the prior art suggest all of the elements of the claimed invention, that there is a motivation to combine the reference teachings, and that there is a reasonable expectation of success at arriving at the claimed invention.

Applicants respectfully maintain that at the time of the invention, one of skill in the art had no motivation to use the *Ac/Ds* system in barley because of the high degree of methylation in the barley genome. Moreover, even if one were to try to use the system in barley, there was no reasonable expectation of success. As explained by Dr. Lemaux in her Declaration (submitted with the response of December 3, 2002) because of methylation and gene silencing in the barley genome, it could not reasonably be expected that the *Ac/Ds* system could generate transformants in which the transposable element can be reactivated and reinserted into the genome nor that the transgene would be expressed.

Although applicants believe that the *prima facie* case of obviousness has been improperly maintained, applicants now provide evidence that there was a long-felt need for

transformation methods that avoid the problems of gene inactivation, particularly in cereals, such as barley. The present invention is surprisingly effective as a means for producing transgenic barley plants that stably express transgenes through multiple generations. It is well established that evidence that an invention provides a solution to a long-felt need in the art can be used to rebut a *prima facie* case of obviousness (*see* MPEP 716.04). An applicant can also present evidence of the surprising effectiveness of the invention to rebut the *prima facie* case. *In re Soni*, 34 USPQ2d 1684 (Fed. Cir. 1995).

The attached Declaration by Dr. Peggy Lemaux and the publication by the present inventors (Koprek *et al., Plant Physiol.* 125:1354-1362 (2001)) provides evidence that prior art gene delivery methods have been plagued by problems of gene inactivation. The Declaration and reference also show that transposase-mediated gene delivery in barley, as claimed here, is surprisingly effective in producing transgenic barley plants with stable transgene expression through generation advance. In particular, as compared to prior art methods, such as biolistics, electroporation and *Agrobacterium*-mediated gene delivery, methods of the present invention result in a greater percentage of single copy insertions', these insertions are much less prone to gene silencing. In addition, unlike prior art methods, transposons preferentially insert in transcriptionally active regions of the barley genome, which further limits the degree of transgene silencing.

As noted by Dr. Lemaux, gene inactivation has been observed in many transgenic plants and has been especially problematic in cereals. Biolistics, the most commonly used method of transformation, leads to complex, multicopy transgene integration, that results in gene silencing in more than 50% of transgenic plants (*see,* Lemaux Declaration, ¶ 5 and Koprek *et al.*, page 1354, first column). Since the successful use of transgenic plants in agriculture requires the development of plants that stably express the transgene during generation advance, there is much interest in developing methods that overcome these problems. Clearly, as of the time Koprek *et al.* was filed, as well as the filing of the present application, these problems had not yet been solved.

The presently claimed methods, however, provide a surprisingly effective means for achieving these goals. As presently claimed, the methods of the invention comprise introducing a *Ds* element containing a desired expression cassette into a barley plant, such that the expression cassette is flanked by the *Ds* inverted repeats. The *Ds* element carrying the transgene reintegrates into the barley plant genome through transposase-mediated excision. Barley plants are then selected in which the *Ds* element has reinserted. The methods of the invention provide an unusually high percentage of transformants with single copy insertions integrated in transciptionally active regions that avoid the problems of gene silencing and thus lead to stable transgene expression (*see*, Lemaux Declaration, ¶ 6 and Koprek *et al* page 1355, first column).

As explained by Dr. Lemaux in ¶ 7 of her declaration, she and her co-authors introduced into plants a *Ds* element carrying a selectable marker gene (*bar* which confers resistance to the herbicide Basta) by particle bombardment. These plants were then crossed with Ac transposase expressing plants. Resulting F_1 plants were selfed and F_2 plants were analyzed to identify plants in which the *Ds* element had transposed and segregated away from the Ac transposase gene. These plants are referred to in the paper as "TNP" plants. Plants which lack transposase genes and in which the *Ds* transposon is thus at the original site of integration are referred to as "nTNP" plants (Lemaux Declaration, ¶ 7).

Table 1 on page 1356 of Koprek *et al.* shows the results of the analysis of TNP and nTNP plants for stable transgene expression through the F_4 generation. There it can be seen that only 6 of 35 (17%) F_3 generation TNP lines had gene silencing, as measured by sensitivity to Basta, whereas transgene expression in 14 of 17 (82%) F_3 generation single and multiple copy nTNP lines was silenced. In the F_4 generation, 7 of 35 (20%) of TNP lines showed gene silencing, compared to 16 of 17 (94%) in nTNP lines. Thus, in the F_4 generation, 80% of TNP lines expressed the transgene, as opposed to only 6 % in the nTNP lines (Lemaux Declaration, ¶ 8).

Comparing only barley plants comprising a single copy of the transgene showed even more stability (Lemaux Declaration, ¶ 9) relative to nTNP plants. Table 1 of Koprek *et al.*

shows that lines derived from F_1 plants A8-1 and A8-5 (both of which have a single *Ds* element) had 84% of TNP lines expressing the transgene in both the F_3 and F4 generations (gene silencing in 4 of 25 or 16%).

These results are also illustrated in Figure 2 of Koprek *et al.*, which shows the large difference in transgene expression (TGE) instability in TNP plants through the F_4 generation, as compared to single and mulitple copy nTNP plants. Thus, these experiments show that transposase-mediated excision and reinsertion dramatically increases the stability of transgene expression from genes embedded in the *Ds* element in barley plants (Lemaux Declaration, ¶ 10).

The unusual stability of the gene expression in TNP plants was investigated by analyzing the integration sites of the transposed *Ds* elements (Lemaux Declaration, ¶ 11). As shown in Figure 3 and discussed in the second column of page 1356 of Koprek *et al.*, the *Ds* elements in these plants had integrated in single to low copy regions of the genome (*i.e.*, transcriptionally active regions of the genome). As stated by the authors at page 1359, second column, a factor that may play a decisive role in the unusual stability of the transgenes in these plants is that 80% of the *Ds* elements re-inserted into single to low copy genomic regions.

As noted by Dr. Lemaux, she and her co-authors conclude the paper by stating that the use of transposable elements in gene delivery "leads to large numbers of independent single-copy transgenic plants." In addition, the increased stability in TNP plants "gives rise to a system of gene delivery that has great utility for generating crop plants with improved agronomic characteristics." (page 1360, first column). The authors could make such a statement only if the reviewers of the manuscript in this prestigious journal were also convinced that this is true.

In conclusion, the methods of the present invention address a long-felt need for efficient methods for producing transgenic barley plants that stably express an introduced transgene. The data discussed above show that the methods of the invention are surprisingly effective in producing such plants. As noted above, the case law is clear that, even assuming a *prima facie* case of obviousness is maintained, evidence that a long felt need was addressed by

the surprising effectiveness of the claimed invention properly rebuts the *prima facie* case. In light of the above, applicants respectfully submit that the rejection should be withdrawn.

CONCLUSION

In view of the foregoing, Applicants believe all claims now pending in this Application are in condition for allowance and an action to that end is respectfully requested. If a telephone conference would expedite prosecution of this application, the Examiner is invited to telephone the undersigned at 415-576-0200.

Respectfully submitted,

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